

## CLAIMS:

- 5 1. A method for identifying and/or obtaining a modulator of a rhomboid polypeptide, which method comprises:
- (a) contacting a rhomboid polypeptide and a substrate polypeptide in the presence of a test compound and one or more non-rhomboid proteases,
- 10 wherein said substrate polypeptide comprises a core domain which has a rhomboid cleavable TMD sequence linked to an upstream tag sequence, the core domain sequence not being susceptible to cleavage by the one or more non-rhomboid proteases, and;
- (b) determining the presence or amount in said medium of a
- 15 soluble polypeptide fragment comprising said tag sequence.
2. A method according to claim 1 wherein said Rhomboid polypeptide and said substrate polypeptide are co-expressed in a cell.
- 20 3. A method according to claim 2 wherein the cell is a mammalian cell.
4. A method according to any one of claims 1 to 3 wherein the presence of the soluble substrate polypeptide is determined by;
- 25 (a) contacting said medium with an specific binding member which binds to said tag sequence, and
- (b) determining binding of soluble polypeptide fragment to said binding member.
- 30 5. A method according to claim 4 wherein said specific binding member is immobilised.
6. A method according to claim 5 wherein said specific binding member is an antibody.

7. A method according to claim 6 wherein said antibody is immobilised on the surface of microtitre plate.

8. A method according to any one of the preceding claims wherein the substrate polypeptide comprises an extracellular detectable label.

9. A method according to claim 8 wherein the label is secreted alkaline phosphatase.

10. A method according to claim 8 or claim 9 wherein the binding of said polypeptide fragment to said anti-tag antibody is detected by determining the amount of said label bound to the antibody.

11. A method according to claim 10 wherein the amount of said label is determined by contacting said label with a reporter molecule which produces a signal in the presence of said label, and measuring said signal.

12. A method according to claim 11 wherein the signal is light emission.

13. A method according to any one of the preceding claims wherein the tag sequence is positioned 10 amino acid residues or less upstream of said TMD in said core domain.

14. A method according to any one of the preceding claim wherein the tag sequence consists of 30 amino acids or less.

15. A method according to any one of the preceding claims wherein the tag sequence is MRGS(H)<sub>6</sub>.

16. A method according any one of the preceding claims wherein the rhomboid cleavage TMD rhomboid comprises a luminal portion which has the same conformation within the membrane as Spitz residues 140-144.

17. A method according to claim 16 wherein the rhomboid cleavable TMD has a luminal portion which comprises or consists of Spitz residues 140-144 (IASGA).

5 18. A method according to claim 16 or claim 17 wherein the rhomboid cleavable TMD is a rhomboid ligand TMD.

19. A method according to claim 18 wherein the rhomboid cleavable TMD is the Spitz TMD.

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20. A method according to any one of the preceding claims wherein the substrate polypeptide comprises a cytoplasmic domain, said domain comprising the cytoplasmic domain of TGF $\alpha$ .

15 21. A method according to any one of the preceding claims wherein the substrate polypeptide comprises a cytoplasmic domain, said domain comprising the cytoplasmic domain of thrombomodulin.

20 22. A method according to any one of the preceding claims wherein the Rhomboid polypeptide has a sequence shown in Table 1.

23. A method according to claim 22 wherein the Rhomboid polypeptide is selected from the group consisting of Drosophila Rhomboid 1, Drosophila Rhomboid 2, Drosophila Rhomboid 3, Drosophila Rhomboid 4,  
25 Human RHBDL-1, Human RHBDL-2 and Human RHBDL-3, E. coli glgG, B. subtilis ypqP, P. stuartii A55862 gene product, P. aeruginosa B83259 gene product, S. cerevisiae YGR101w and S. cerevisiae YPL246c.

24. A method according to any one of the preceding claims  
30 comprising identifying said test compound as a modulator of Rhomboid protease activity.

25. A method according to claim 24 comprising isolating said test compound.

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26. A method according to claim 25 comprising synthesising and/or preparing said test compound.

27. A method according to claim 25 or claim 26 comprising modifying  
5 said compound to optimise the pharmaceutical properties thereof.

28. A method according to any one of claims 24 to 27 comprising  
formulating said test compound in a pharmaceutical composition with  
a pharmaceutically acceptable excipient, vehicle or carrier.  
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29. A modulator of Rhomboid protease activity obtained by a method  
of any one of claims 1 to 23.

30. A method of making a pharmaceutical composition comprising,  
15 identifying a compound as a modulator of Rhomboid activity  
using according to any one of claims 1 to 23,  
synthesising, preparing or isolating said compound and  
admixing the compound with a pharmaceutically acceptable  
excipient, vehicle or carrier, and optionally other ingredients to  
20 formulate or produce said composition.

31. A method according to claim 30 comprising modifying said  
compound to optimise the pharmaceutical properties thereof.

25 32. A method according to claim 30 or claim 31 comprising  
determining the activity of a Rhomboid polypeptide in the presence  
of said composition.

33. A polypeptide which is proteolytically cleavable by a Rhomboid  
30 polypeptide, said polypeptide comprising an a core domain which has  
a rhomboid cleavable TMD sequence linked to an upstream tag  
sequence, the core domain sequence not being susceptible to cleavage  
by mammalian metalloproteases.

34. A polypeptide according to claim 33 wherein the tag sequence is positioned 10 amino acid residues or less upstream of said TMD in said core domain.

5 35. A polypeptide according to claim 33 or 34 wherein the tag sequence consists of 15 amino acids or less.

36. A polypeptide according to claim 35 wherein the tag sequence is MRGS(H)<sub>6</sub>

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37. A polypeptide according to any one of claims 33 to 36 wherein the rhomboid cleavage TMD rhomboid comprises a luminal portion which has the same conformation within the membrane as Spitz residues 140-144.

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38. A polypeptide according to claim 37 wherein the rhomboid cleavable TMD has a luminal portion which comprises or consists of Spitz residues 140-144 (IASGA).

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39. A polypeptide according to claim 37 or claim 38 wherein the rhomboid cleavable TMD is a rhomboid ligand TMD.

40. A polypeptide according to claim 39 wherein the rhomboid cleavable TMD is the Spitz TMD.

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41. A polypeptide according to any one of claims 33 to 40 wherein the substrate polypeptide comprises an extracellular domain, said domain comprising a detectable label.

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42. A polypeptide according to claim 41 wherein the label is secreted alkaline phosphatase.

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43. A polypeptide according to any one of claims 33 to 42 wherein the substrate polypeptide comprises a cytoplasmic domain, said domain comprising the cytoplasmic domain of thrombomodulin.

44. An isolated nucleic acid encoding a chimeric polypeptide according to any one of claims 33 to 43.

5 45. An expression vector comprising a nucleic acid according to claim 44.

10 46. A host cell comprising an expression vector according to claim 45 or a chimeric polypeptide according to any one of claims 33 to 43.

47. A host cell according to claim 46 further comprising an expression vector comprising a nucleic acid encoding a rhomboid polypeptide.

15 48. A method for obtaining a cleavage product of a Rhomboid polypeptide, which method comprises:

(a) contacting a Rhomboid polypeptide and a substrate polypeptide and one or more non-rhomboid proteases,

20 wherein said substrate polypeptide comprises a core domain which has a rhomboid cleavable TMD sequence linked to an upstream tag sequence, the core domain sequence not being susceptible to cleavage by the one or more non-rhomboid proteases, and;

(b) contacting said medium with an antibody which binds to said tag sequence, and

25 (c) isolating/purifying soluble polypeptide fragment bound to said antibody.

49. A method according to claim 48 comprising sequencing the polypeptide fragment.

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